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1961

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A FACTOR INACTIVATING SUBSTANCE P PRESENT IN THE SERUM OF PREGNANT WOMEN

Ever since the discovery of SP, its destruction by specific or unspecific enzymes has been a question of major interest. Such an interest is fully justified in view of the fact that SP is a physiological product, belonging probably to the group of biologically active polypeptides which undergo destruction by specific enzymes. We have reason enough to believe that SP is chemically related to oxytocin and vasopressin, though recent results by Franz, Boissonnas and Stürmer (1961) do not seem to support our opinion. Since SP is produced in tissues rich in oxytocin and vasopressin which in turn, interact with specific enzymes, it seems reasonable also to assume the existence of an enzyme specifically inactivating SP.

The inactivation of SP by various tissues has been well known for a long time. Gaddum and Schild (1934), as well as von Euler (1934) have reported it many years ago. Subsequently Pernow (1955), Arvidsson et al. (1956), Lembeck and Eber (1956) and Matussek (1959) dealt with inactivation of SP in detail. Arvidsson, Pernow and Swedin (1956) have expressed the opinion that there must be a specific SP-ase. These authors succeeded in separating an SP inactivating component from diaminooxydase.

In the present work we propose to answer two basic questions: firstly, whether the quantity of the SP-inactivating factor which, according to Lembeck (1956), is present in normal serum (NS) at a low level, increases during pregnancy (pregnant serum, PS), and secondly, whether this factor can be differentiated from diaminooxydase, oxytocinase and vasopressinase by specific inhibitors. The latter question is obviously very significant in judging the astructural relationship of SP and the posterior pituitary hormones. Moreover it is known that the enzymes specifically inactivating these hormones also occur at high levels in PS (Tuppy, 1960).

Method

The SP used had a potency of 6—8 U./mg. Testing was performed by the usual procedure using the isolated guinea pig intestine. The

enzyme preparation (PS) was made from retroplacental blood taken immediately after parturition and centrifuged at once. The PS used was never older than 24 hours. NS was obtained from healthy, non-pregnant women, and used within 24 hours. The following inhibitors have been used: aqueous aminoguanidine, 0.075 mg/ml, for diamino-oxydase (Schuler, 1952); p-chloromercuribenzoate (PCM), 0.38 mg/ml, for oxytocinase and vasopressinase (Werle, 1960) and the tripeptide S-benzyl-L-cysteinyl-L-leucyl-glycinamide (S-L), 0.0735 mg/ml, specifically for oxytocinase. We have included the examination of the effects of patulin, LSD, and SKF-525A on GS activity in view of the well known potentiation of the action of SP on isolated intestine by LSD (Werle and Effkemanon, 1940), and the inhibition of biogenic amines, H, 5-HT and ACh, but not SP, by patulin (Eliasson, 1958). Since we have found earlier that SKF-525A prolongs the action of SP (Stern and Dobrić, 1957), we also examined this compound.

1. series

5 mg SP was added to both 1 ml NS and 1 ml PS in order to see whether GS inactivates SP more strongly than NS.

2. series

Aminoguanidine was added to NS and PS to test whether the elimination of diaminooxydase influences the destruction of SP.

3. series

PCM was added to NS and PS in order to ascertain whether elimination of oxytocinase and vasopressinase influences the destruction of SP.

4. series

Aminoguanidine and PCM were added simultaneously to see whether inactivation of all three enzymes, diaminooxydase, oxytocinase and vasopressinase have any influence on the destruction of SP.

5. series

PS was treated with LSD (10^{-4} and 10^{-5} g/lit.)

6. series

PS was treated with patulin (10^{-4} and 15^{-5} g/lit.)

7. series

PS was treated with SKF-525A (10^{-5} g/lit.).

Results

Inactivation of SP by NS is weaker than that by PS (Fig. 1). PS is generally 5 to 6 times as active as NS, in some experiments even more than that. There is a distinct time-dependence; the decrease of contractions is obvious after 15, 30, and 60 min. incubation. Elimination of diaminooxydase has no effect upon the rate of SP inactivation (Fig. 2); it is to be recalled that PS is very rich in diaminooxydase (Werle, Effkemanon, 1940). Neither the elimination of oxytocinase by S-L, nor that of both, oxytocinase and vasopressinase by PCM influences the

FIG. 1

Contractions of guinea pig ileum produced by SP treated with normal serum (NS). SP was previously incubated with Tyrode's solution for 10 min. (control) (1), and with NS for 10, 30, and 60 min., respectively (2, 3, and 4).

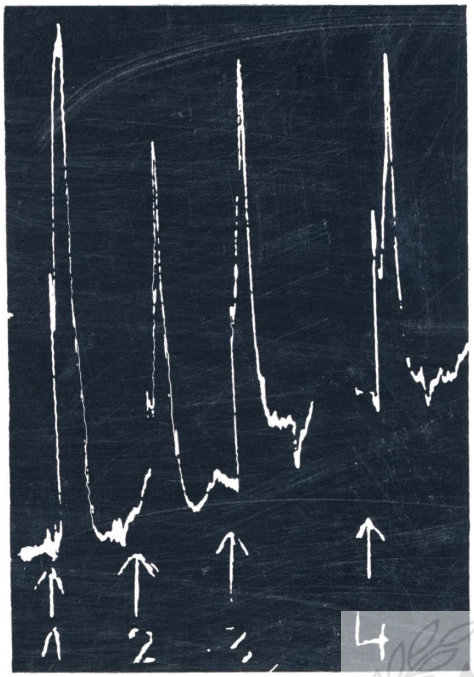


FIG. 2

Contractions of guinea pig ileum produced by SP treated with pregnant serum (PS). The experiment was performed as in previous Figure using PS instead of NS.



FIG. 3

Contractions of guinea pig ileum produced by SP and H treated with PS. Addition of SP, control (1), previously incubated with PS, alone (3), and PS in presence of aminoguanidine (5); addition of H treated in the same manner (2, 4, and 6).



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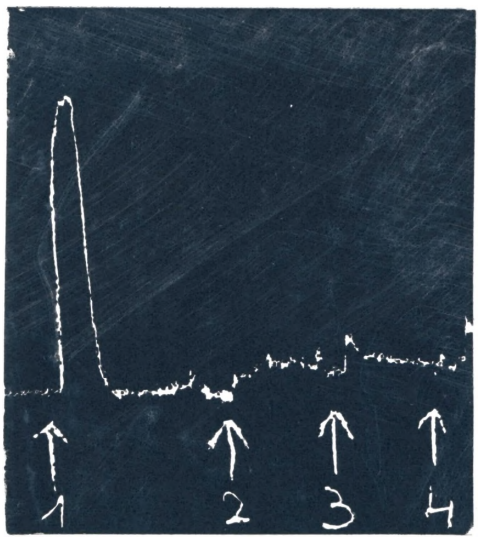


FIG. 4

Contractions of guinea pig ileum produced by SP treated with PS in presence of inhibitors. Addition of SP. control (1), previously incubated with PS, alone (2), in presence of PCM (3), and in presence of PCM and aminoguanidine (4).

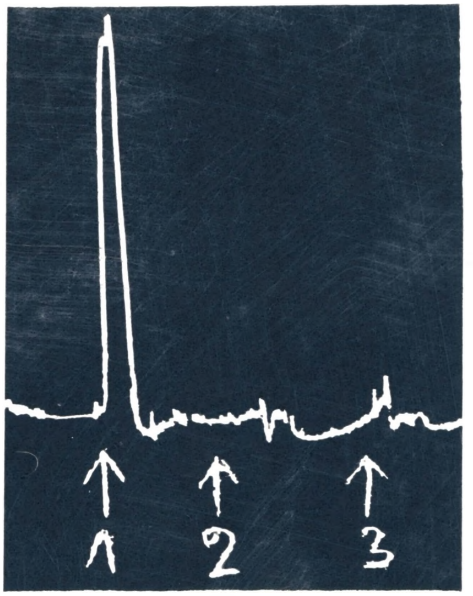


FIG. 5

Influence of patulin on inactivation of SP by PS. SP was previously incubated with Tyrode's solution for 10 min. (control) (1), and with PS in presence of patulin for 30 and 60 min., respectively (2, and 3).

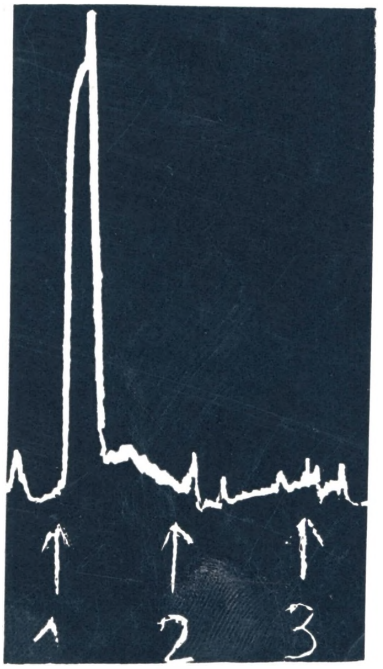


FIG. 6

Influence of SKF 525A on inactivation of SP by PS. Experimental conditions same as in previous Figure.

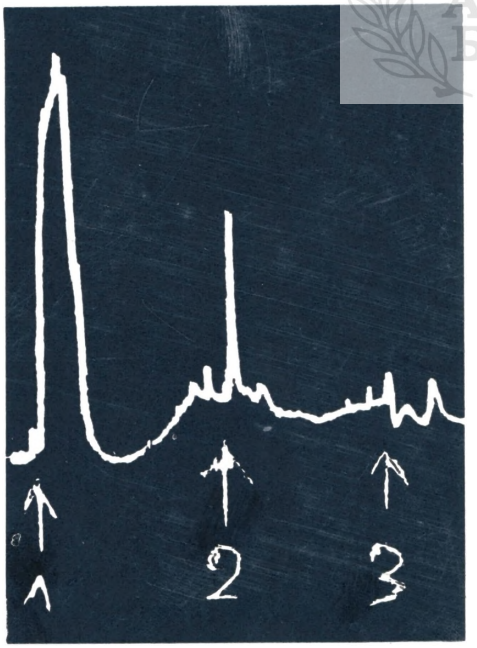


FIG. 7

Influence of LSD on inactivation of SP by PS. Experimental conditions same as in two previous Figures.

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destruction of SP by PS (Fig. 3). [It should be mentioned here that, according to Werle (1960), the vasopressinase activity of NS equals that of PS, whilst oxytocinase activity is markedly greater in PS.] The simultaneous elimination of diaminooxydase, oxytocinase and vasopressinase, likewise, has no effect on SP-ase activity (Fig. 4). Finally, as can be seen from Fig. 5, LSD, patulin and SKF-525A do not inhibit the inactivation of SP by PS. When LSD was added to PS prior to incubation with SP, there was still some SP activity left after 30 min., but after 60 min. (Fig. 6 and 7) complete inactivation occured. We are not able to tell at this moment whether this observation is due to a reduction of the rate of inactivation by LSD, or is it a consequence of the periphereal potentiation of the SP-activity in the isolated intestine by LSD as decribed by Krivoy (1957).

Discussion

It is to be concluded from these experiments that human serum contains an enzyme inactivating SP which is different from diaminooxydase, oxytocinase and vasopressinase. The enzymatic activity increases considerably during pregnancy. According to Matussek (1959) diaminooxydase isolated from the pea inactivates SP even in presence of aminoguanidine. We have obtained entirely different results with PS, which in presence of aminoguanidine inactivates SP, but not H. The difference between Matussek's findings and our own are probably due to the different sources of the SP-inactivating factor.

Arvidsson, Pernow and Swedin (1956), working with a diaminooxydase isolated from the kidney, were able to separate therefrom a factor inactivating SP. This speaks in favour of our own conclusions.

From our results it is impossible to tell what kind of enzyme performs the inactivation of SP. We only wish to stress that chymotrypsin destructs SP at a rate 200 times that of trypsin, a fact supporting the assumption about the polypeptidic character of SP. Krivoy (1957) observed that LSD inhibits a factor from guinea pig brain inactivating SP, but does not inhibit chymotrypsin. This finding was confirmed by Smith and Walaszek (1961). These authors found that the SP-inactivating factor from guinea pig ileum is likewise not inhibited by LSD, and that eserine potentiates the effect of SP on the gut. Since chymotrypsin is an esterase we could suppose that the SP-inactivating factor in PS is identical with chymotrypsin, and that in the guinea pig brain it is different from chymotrypsin. We would like to add that, according to Krivoy (1957), the guinea pig brain extract does not inactivate oxytocin, the same as we found for the factor from PS. The fact that patulin, acting spasmolytically by itself, and that SKF-525A do not influence the rate of inactivation of SP by PS, shows that SP-ase is not involved in the respective effects of these drugs on the action of SP. SKF-525A prolongs the tranquillizing effect of SP wich is a central one; we have not investigated the action of SKF-525A on the SP-inactivating factor in the brain. In connection with this problem it

is interesting to point out the results obtained by Kocić-Mitrović (1961), who found that the concentration of SP is lowered in the uterus and brain of pregnant rats, and the SP-ase activity in their serum is increased. Therefore the question arises whether SP plays some rôle in the mechanism of parturition having in mind that it produces contraction of smooth muscles in the uterus. Alternately, this rôle of SP could be deduced from the fact that increased SP-ase activity during pregnancy has the task of preventing contractions of the uterus by destructing excess SP, the same as it is the case with oxytocin and H.

We should mention the fact that already a few hours after parturition the SP-ase activity in the serum decreases. Even before parturition this activity is much less than in retroplacental serum.

Our results show that the serum of pregnant women contains a factor inactivating SP, which is not identical with diaminooxydase, oxytocinase and vasopressinase, and cannot be inhibited by LSD.

Summary

In retroplacental and pregnant serum there is an agent destroying SP. This agent is also found in the serum of normal women, but in much smaller quantities. The agent could not be inhibited by specific inhibitors of diaminoxydase, oxytocinase or vasopressinase. Pregnant serum incubated with SKF 525A enhanced the contractions of the isolated guinea pig ileum. Probably the agent is an enzyme similar, perhaps, to chymotrypsin.

FAKTOR SERUMA TRUDNICE KOJI INAKTIVIRA SUPSTANCIJU P

U retroplacentarnom serumu, a i u serumu gravidne žene, nalazi se neki agens koji razara SP. Ima ga i u normalnom serumu žene, ali u mnogo manjim količinama. Ovaj se faktor ne da inhibirati inhibitorima diaminooksidaze, oksitocinaze ili vazopresinaze. Retroplacentalni serum inkubiran sa SKF 525A pojačava kontrakcije izolovanog crijeva zamorca. Vjerojatno je da se radi o fermentu, koji je, možda, sličan kimotripsinu.

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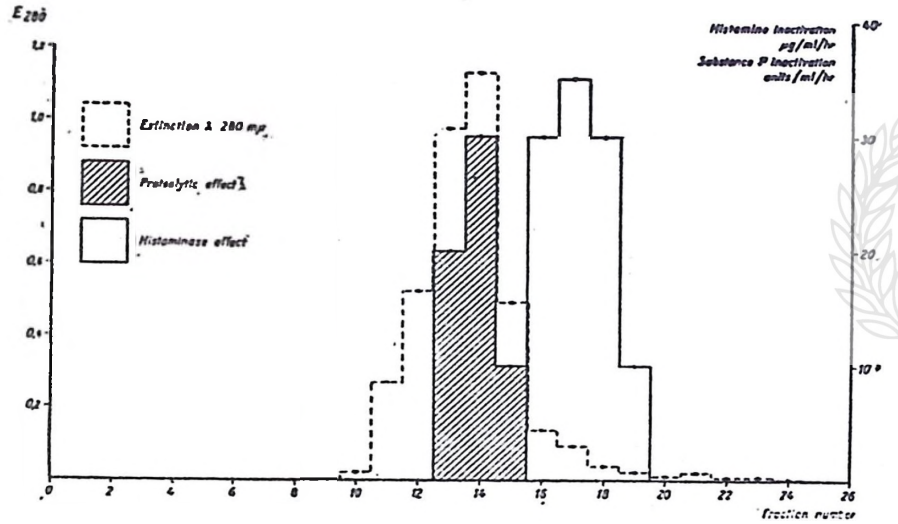
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DISCUSSION

PERNOW: As was mentioned in the paper, Arvidsson, Swedin an I (Acta physiol. scand. 1956, 35, 338) found that the proteolytic principle from kidney extracts, which inactivates SP was easily separated from the histaminolytic factor. The separation shown in the Figure was done on an ion exchange column (Dowex 50) using M/50 phosphate buffer, pH 7.1.



Separation of the histaminase and the substance P-inactivating agent in kidney extract on an ion exchange column (Dowex 50, 0.9 × 20 cm) in a M/150 phosphate buffer, pH 7.1. Effluent volume was 0.3 ml per 30 minutes.

Has anybody tried to extract SP from blood or urine?

STERN: Probably there is no SP in the blood. As far as I am aware there have been no attempts to extract SP from urine.

ZETLER: Matussek has found that diamine oxydase destroys SP. How do you explain the discrepancy to your findings?

STERN: Matussek extracted the enzyme from pea. We used the enzyme from human pregnant serum. We think that the different provenience of the enzymes might be the reason for this disagreement.